

Comparing Astringency and Sourness of Whey Protein Beverages Acidified with Four  
Different Acids

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Abby M. Dornbusch

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## **ABSTRACT**

Increasing interest in whey protein beverages stems from the wide range of nutritional benefits whey proteins have to offer. A useful characteristic of whey proteins is their solubility over a wide pH range, however in order to ensure clarity of a ready-to-drink whey protein beverage, it needs to be manufactured at a pH of approximately 3.4. At an acidic pH of 3.4, the beverages become astringent and can lead to consumer acceptability issues. The main objective of this research was to determine which, if any, of four different acids (hydrochloric, malic, phosphoric, tartaric) achieved the lowest perceived astringency rating when used to acidify a 4% (w/v) whey protein isolate (WPI) solution to pH 3.4. A secondary objective was to identify the buffer capacities of each acid in both a water solution and WPI solution, in efforts to detect a relationship between buffer capacity and perceived astringency. Sourness ratings for each sample were also gathered. A 4% (w/v) WPI solution acidified with hydrochloric acid generated the lowest perceived astringency and sourness ratings. Conversely, the malic acid WPI sample produced the highest perceived astringency rating. Additionally, hydrochloric and phosphoric acid samples buffered the least within the pH range of interest (3.4-7.0). This research indicates a potential relationship between the perceived astringency of an acidified-WPI solution and the buffer capacity of the acidulant used.

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## **Introduction**

### **Importance of Whey Protein**

One of the fastest growing segments of the beverage industry is nutritional or functional beverages; sales have grown 48% from 2008 to 2013 (Bloom, 2014). Included in the wellness/functional beverage category are dairy-based beverages, nutrient-enhanced drinks and protein-enhanced beverages. The steep growth of this category is attributed to the consumer's need for convenient nutrition, increasing health concerns and desire to lead a healthy lifestyle. Consumers are looking to ready-to-drink, protein-rich beverages to become physically fit and to lose weight (Bastian, 2004). Consequently, manufacturers of wellness beverages are looking to use ingredients that deliver on the consumer's evolving health-related needs (Miller, 2005). One such ingredient is whey protein. Recent interest in whey protein beverages stems from the wide range of nutritional benefits whey proteins have to offer. Whey protein is considered a high-quality protein because it contains a variety of branched-chain essential amino acids required by the human body, most notably leucine which is believed to promote muscle protein synthesis (Miller, 2005). Whey protein isolate contains ~10.9% leucine, whereas other protein sources such as soy protein isolate and egg whites contain ~8.0-8.8% leucine (Norton et al., 2012). Additionally, calcium and minerals found in whey products may increase weight loss during energy restriction by facilitating lean tissue formation (Ha & Zemel, 2003).

### Functionality of Whey Protein

A useful characteristic of whey proteins is their solubility over a wide pH range. Whey proteins are typically used in beverages that are fruit-flavored and acidic with a pH ranging from 2.8-4.5, or in beverages that are neutral and shake-like with a pH ranging 5.5-7.0. Both applications provide a source of whey protein; however, the acidic, fruit-flavored beverages need to be manufactured at a pH of approximately 3.4 in order to ensure clarity of the beverage and heat stability. While the acidification of the beverages inhibits protein denaturation and precipitation, it impacts the sensory profile by increasing the astringency of the product (Beecher et al., 2008). Increases in astringency sensations can be problematic in acidified whey protein beverages, as they can cause consumer acceptability issues (Childs & Drake 2010).

### Consumer Acceptability of Acidified WPI Beverages

Childs and Drake (2010) conducted an interesting study to understand consumers' perceptions of astringency and how it may impact their acceptability of an acidified-protein beverage. Consumers participated in focus groups and liking tests and were given a variety of acidified-protein beverages to taste, each containing different amounts of protein. All beverages were tasted with and without nose clips in order to understand the impact of orthonasal factors on the consumers' perceptions of the beverages. Popular beverages that do not contain protein, such as cranberry and grape juices, were found astringent, but the astringency did not impact the consumer's acceptability of the product. During focus groups, consumers explained they expected these popular products to be

astringent and enjoyed the flavors of the beverages; therefore the astringency didn't affect their acceptance of the product. When the same consumers tasted acidified-protein beverages containing whey protein isolate (WPI), they generally did not like the beverages and cited astringency, off-aromas and off-flavors as reasons. The results from the consumer liking test showed that when acidified WPI beverages were tasted without nose clips, overall liking scores decreased as protein concentration increased; however when the same samples were tasted with nose clips, overall liking scores were almost unaffected by increasing protein concentration. This contrast indicates that orthonasal factors are the primary source of low consumer acceptability of acidified WPI beverages. Consequently, this study provides sound evidence that flavors inherent to WPI, and most likely milk proteins in general, cause the greatest obstacle to consumer acceptance, exacerbated to some extent by astringency.

#### Definition & Mechanism of Astringency

Astringency is defined as a complex group of sensations involving dryness, roughness of oral surfaces and tightening, drawing or puckering of the mucosa and muscles around the mouth (Lee & Lawless, 1991). This tactile sensation can be experienced after consuming foods such as red wine and particular fruits and teas (Breslin, 1993). Since the increased popularity of protein-enriched drinks, acidified whey protein beverages can also be included in this list as an astringency-producing food. Astringency has been gaining attention by dairy food manufacturers, and efforts to understand and reduce this drying sensation have increased.

Astringency is a complex sensation and understanding the mechanism behind it differ depending on the type of compound producing the astringency. There are four known groups of compounds that contribute to astringency: acids, metal salts, polyphenols and dehydrating agents (Green, 1993). Polyphenols, specifically tannins, have been of particular interest as tannins, which are plant-based, can be found in tea, coffee and red wine, three beverages with global popularity. Consequently, much of the astringency research to-date has focused on investigating the contribution of polyphenols to the perception of astringency. More recently the research has expanded to acids and proteins. It has yet to be determined whether mechanisms for astringency caused by polyphenols can be translated to acid and/or whey protein beverage astringency; however, a number of researchers have attempted to establish whether this relationship exists.

The widely accepted model for polyphenol astringency is described as the complex formation of polyphenols and salivary proline-rich proteins (PRPs). After the binding of the polyphenols to the PRPs, the PRPs precipitate out, therefore altering the mucosa layer in the mouth and reducing the saliva's lubricity (Clifford, 1997) (Jöbstl et al., 2004). Sano et al. (2005) proposed a similar mechanism for whey protein-associated astringency, asserting that when an acidic whey protein beverage is consumed (~pH 3.5), the neutral pH of saliva raises the pH of the protein solution. As the pH of the protein-saliva mixture increases, whey proteins precipitate out in the mouth as their isoelectric

point is reached (~pH 5.0). Sano et al. (2005) states that the precipitated whey proteins act as the precipitated PRPs do, increasing the friction in the oral cavity. Beecher et al. (2008) also suggested a mechanism similar to that of polyphenol-based astringency, stating that the salivary and whey proteins interact to form an aggregate, much like the interaction of polyphenols and PRPs, thereby reducing the lubricity of the saliva. Beecher also explained that the effect of pH on astringency depends on the compound that is the source of astringency and, consequently, the mechanism behind it. Both of the mechanisms proposed by Beecher (2008) and Sano et al. (2005), feature whey proteins as the cause of the astringency, either by precipitating out of the solution or by aggregating with PRPs.

In 2008, Lee proposed an alternative explanation for the astringency of acidified whey protein beverages, claiming that astringency is actually caused by the acid and not the whey proteins. Lee's study involved two whey protein solutions (1% and 6% w/v) that were acidified to a pH of 3.4 using phosphoric acid. Two additional samples of diluted phosphoric acid, no whey protein, were tested. These samples matched the acid concentration of the two WPI samples, and consequently had a much lower pH due to the WPI buffering in the other samples. Panelists found the acid-only samples more astringent than the samples containing WPI, implying that the presence of WPI actually decreased the astringency when acid concentration remained constant. Acid is a known astringent, and although the mechanism behind acid-based astringency is not fully defined, researchers have shown that in a buffered solution, salivary proteins can be

precipitated by acids. In 2012, Lee conducted another study to determine whether acid-related astringency was indeed caused by the precipitation of PRPs and whether the oral mucus layer was affected by astringency compounds. Acids did not appear to precipitate PRPs, but they did precipitate non-PRP proteins. Lee (2012) hypothesized that the loss of any salivary protein could decrease the saliva's lubricity; however, the relationship between decreased lubricity and increased astringency was also studied by Lee and showed no correlation. Consequently, it appears that astringency of acidified whey protein beverages is primarily caused by the acid. The mechanism by which the acid causes the astringency is unknown, but is not believed to be related to precipitated PRPs or decreased salivary lubricity.

#### Acids Producing Astringency

In a free-choice profiling of 15 common organic and inorganic acids, astringency proved to be a defining characteristic of the acids. In fact, at the concentration tested (0.08% w/v), the inorganic acids were found to be more astringent than they were sour (Rubico & McDaniel, 1992). The authors declared that, "an expanded mechanism or set of mechanisms for astringency that takes into account not only tannin-protein interactions but also acid-protein interactions, must be studied." An additional study was conducted several years later; however, that study focused on fewer acids, and unlike the previous study, pH was tested at three levels but kept constant amongst all the acids. Again, astringency was found to be a defining attribute of the acids; however, a new finding showed differentiation in astringency amongst the samples at pH 3.5. In fact, at this pH

the lactic acid sample displayed the most astringency. Also, panelists found the samples at pH 4.5 to be less astringent than the samples at pH 3.5 or 6.5 (Hartwig & McDaniel, 1995). Lawless, Horne & Giasi (1996) expanded on the potential relationship between pH level and astringency perception by testing three acids at three different pH levels. Panelists rated attributes for each sample on a 15-point scale. A relationship between pH and astringency was found, showing that as pH increases, astringency decreases. The results from these three studies are in agreement with Lee's (2008) findings that at the pH necessary to clarify whey protein beverages (~3.4), acids are largely contributing to the overall astringency of the beverage.

#### Mechanism of Sour Taste

Much like astringency, the mechanism for sour taste is not yet fully understood. Ganzevles & Kroeze (1987) proposed two separate mechanisms contributing to sour taste perception: one for weak, organic acids and another for strong, mineral acids. Because strong mineral acids are fully dissociated at any pH, sourness is believed to be directly linked to the presence of hydrogen ions which interact with the epithelium, most likely through a proton channel. Weak, carboxylic acids on the other hand are only somewhat dissociated; therefore it is thought that these molecules interact at a receptor site, separate from that of strong acids. DeSimone (2001) theorized that weak acids enter taste receptors through a lipid bilayer by process of diffusion. He also asserted that, for both strong and weak acids, sourness is ultimately caused by a decrease in intracellular pH in acid-sensing taste receptor cells. CoSeteng et al. (1989) stated more generally that

sourness perception is a function of dissociation constants, anion concentration and the chemical structure of the acid.

CoSeteng et al. (1989) asserted that perceived sourness among acids depends on both molecular weight and polarity; the lower the molecular weight and stronger the polarity, the less perceived sourness. Additionally, weak acids show varying intensities of sourness based on their number of carboxylic groups; the more carboxylic groups, the lower the sourness intensity (CoSeteng et al, 1989); therefore in the study presented here, it would be expected that a solution containing hydrochloric acid would have the lowest perceived sourness, followed by a solution containing phosphoric acid, then malic acid and lastly tartaric acid.

#### Buffer Capacity

A solution buffers when it is resistant to a change in pH in the presence of a strong base or acid. The buffer capacity of a solution at any given time is defined as the ability of that solution to resist a change in pH. The greater the buffer capacity of a solution, the smaller is the observed change in pH. When a food product is being formulated, special attention needs to be paid to the buffering capacity of the product's ingredients. If an ingredient or combination of ingredients buffers at too high or too low pH, the desired taste, aroma, texture and appearance of the product can be affected. Buffer capacity was examined in this study because astringency and sourness can both be linked to a product's buffer capacity. If a solution buffers at or near the final desired pH, increased



amounts of acids or base will need to be added in order to reach the desired pH.

In the specific case of low-pH whey protein beverages, which was examined in this study, buffer capacity can be problematic because as increased amounts of acid need to be added to the product, sourness and astringency are amplified, which can affect consumer acceptance of the product. As previously mentioned, to clarify a whey protein beverage, acid must be added to reduce the pH of the WPI solution from neutral to around 3.4. As the pH drops from ~7.0 to ~3.4, buffering takes place. Buffering is highest between a pH of 4.0 and 3.0, where whey proteins have maximum buffering capacity. Whey protein is comprised of amino acids, some of which are acidic; therefore the high buffering capacity of WPI is a summation of the amino acids' buffering capacities (Kailasapathy, Supriadi & Hourigan, 1996; Metwally & Awad, 2001; Srilaorkul, Ozimek, Wolfe & Dziuba, 1989). In this study the buffer capacities of four acids (phosphoric, hydrochloric, malic and tartaric) were experimentally determined, as well as the buffering capacities of 6% WPI solutions titrated with each of the four acids. In order to reduce the sourness and astringency of the final product, the amount of acid added to the WPI solution needs to be reduced. Therefore, by determining the buffering capacities of each WPI solution and each individual acid, the acid that contributes least to the buffering between pH 3.4-7.0 can be identified.

### Objectives & Hypotheses

In this study, we hypothesized that levels of astringency in acidic whey protein beverages

may differ depending on the type of acid used when clarifying the beverage. More specifically we hypothesized that the acid exhibiting the largest buffer capacity between pH 3.4 and 7.0, would lead to higher astringency ratings, compared to the other acids, when used in a whey protein beverage. One objective of this study was to determine whether the astringency and sourness of an acidic, 4% whey protein isolate (WPI) solution would be affected by changing the specific acid used (i.e. malic, phosphoric, hydrochloric or tartaric). An additional objective was to measure the buffer capacities of each acid in both a water solution and a WPI solution. The buffer capacity for each acid was compared to the sourness and astringency ratings of the corresponding WPI samples.

### **Sourness & Astringency**

#### **Materials & Method**

**Subjects.** Thirty panelists (25 females, 5 males) participated in this study based on their availability and absence of food allergies. All panelists were recruited through the University of MN-Sensory Center database. All sessions were held at the Sensory Center located in the Food Science and Nutrition Building on the St. Paul Campus of the University of Minnesota. The University of Minnesota Institutional Review Board approved all the sensory testing procedures used in this research.

**Training.** A training session was given prior to the first test session in order to familiarize panelists with the concept of astringency and to practice the sample evaluation procedure. The procedure used closely followed that used by Lee & Vickers (2008).

During the training session, panelists first were given the definitions of sourness and astringency. The following definitions of astringency and sourness were used:

*Astringency is a tactile sensation felt in the mouth that is commonly described as a drying-out or roughing sensation. It is experienced on the oral surfaces inside the mouth including the cheeks and inner lip areas, and it is often a delayed sensation taking some time to fully develop.*

*Sourness is the tart and tangy taste. While astringency is a tactile sensation, sourness is a taste.*

Panelists were also given a handout outlining the sampling procedure they needed to follow when tasting each sample. The sampling procedure developed by Lee & Vickers (2008) is outlined below in Table 1.

**Table 1. Sampling Technique (Lee & Vickers, 2008)**

<b>Please follow this procedure for all samples:</b>
<b>1.Place contents of entire 10 ml sample in mouth</b>
<b>2.Rate the sourness of the sample</b>
<b>3.Gently swish the sample in your mouth for 10 seconds</b>
<b>4.Expectorate the sample</b>
<b>5.Silently mouth the word ‘why’ 3 times in an exaggerated movement</b>
<b>6.Rate the astringency of the sample</b>
<b>7.Rinse mouth thoroughly with water until astringency has returned to a level of zero.</b>
<b>IMPORTANT – Do NOT continue to next sample until perceived astringency is zero!</b>

Because astringency is a tactile sensation, it cannot be experienced until one moves their mouth and tongue. Sourness, however is a taste and can be identified simply by holding a sample on one’s tongue, consequently the sampling procedure listed in Table 1 prompts

panelists to first hold the sample in their mouth and rate the sourness before moving the sample around in their mouths. After swishing the sample in their mouths and expectorating, panelists were instructed to silently mouth the word 'why' three times in an exaggerated fashion. This step acted to standardize the way each panelist was assessing the tactile effect the sample had on their mouths.

As a group, the panelists tasted four samples to practice the sampling technique and to discuss the differences between sourness and astringency. One of the four samples was sour containing dilute citric acid (Sigma-Aldrich Co., St. Louis, MO, USA), sucralose (Splenda<sup>®</sup>, Tate & Lyle, Decatur, IL, USA) and flavoring (raspberry flavoring, Polak's Frutal Works, Inc., Middletown, NY, USA), one was astringent containing dilute alum (alum, Barry Farm, Wapakoneta, OH, USA), sucralose and flavoring, and the remaining two samples were both sour and astringent containing WPI (WPI, Instantized BiPro<sup>®</sup>, Davisco, Eden Prairie, MN, USA), acid, water, sucralose and flavoring. In order to distinguish sourness from astringency, panelists first tasted the sour sample followed by the astringent sample. Next, in order to train the panelists to identify both attributes in a single sample, they tasted the two samples which were both sour and astringent. During this portion of the training session, panelists were able to discuss the ratings they gave the samples and ask any questions they had regarding the sampling procedure or the differences between sourness and astringency.

After tasting samples as a group and practicing the sampling procedure, panelists

individually practiced rating the sourness and astringency of four more samples using SIMS 2000 computerized data collection program (Sensory Computer Systems, Morristown, NJ, USA). For this portion of the training session, panelists were given two WPI samples (WPI + acid+ water + sucralose + flavoring) and two acid-control samples (acid+ water + sucralose + flavoring). The purpose of this exercise was to give panelists more practice at deciphering sourness and astringency in the samples, to further practice the sampling procedure, and to familiarize themselves with the computerized data collection program. The researcher was available during the entire training session to answer any questions regarding any portion of the test.

**Test Samples.** Nine samples were evaluated in this study, four test samples, four acid control samples and one phosphate control sample (Table 2).

**Table 2. Test sample formulas and pH values**

Sample ID	Sample Description	Whey (g)	Water (ml)	Acid (ml)	Base (ml Na <sub>2</sub> HPO <sub>4</sub> )	pH
H4	4% WPI with HCl	40	920	80	0	3.4
H	HCl Control	0	920	80	0	1.4
P4	4% WPI with Phosphoric Acid	40	931	70	0	3.4
P	Phosphoric Acid Control	0	931	70	0	1.8
T4	4% WPI with Tartaric Acid	40	913	87	0	3.4
T	Tartaric Acid Control	0	913	87	0	2.3
M4	4% WPI with Malic Acid	40	867	133	0	3.4
M	Malic Acid Control	0	867	133	0	2.2
PC	Phosphate Control	0	660	56	284	3.4

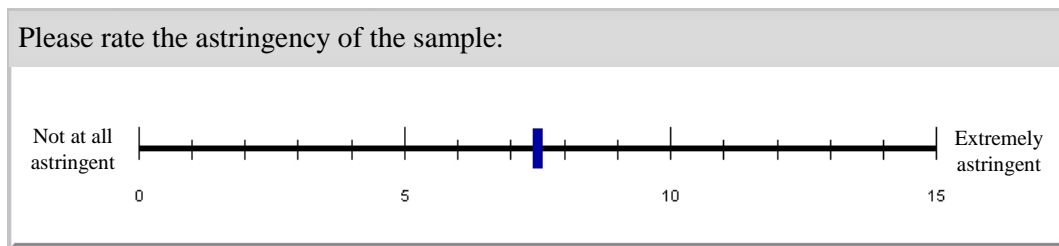
The four test samples contained whey protein isolate. The WPI samples were prepared

by dissolving WPI in distilled water at 4% (w/v) and then titrated using one of the following four acids which were all prepared at a molarity of 0.5: hydrochloric (Fisher Chemical, Fair Lawn, NJ, USA), tartaric (SAFC Supply Solutions, St. Louis, MO, USA), malic (Sigma-Aldrich Co., St. Louis, MO, USA) or phosphoric (Fisher Chemical, Fair Lawn, NJ, USA), until a pH of 3.4 was reached. Measurements of pH were taken using a Corning model 340 digital pH meter (Lowell, MN, USA) and a calomel electrode. The acid control samples were prepared by adding the respective volume of acid used to titrate each of the test samples, to distilled water; the acid control samples did not contain any WPI; however, they did have the same acid concentrations as their respective test sample. The acid control samples were at different pH levels than the test samples. Additionally a phosphate control sample (PC) was included in the test to investigate how phosphates contribute to astringency. The phosphate control was prepared by titrating a disodium phosphate and distilled water solution with phosphoric acid (phosphoric acid, Fisher Chemical, Fair Lawn, NJ, USA) until a pH of 3.4 was reached. The number of moles phosphate in PC was equivalent to that in P4 and P. Samples containing only acid, no WPI, and matching a pH of 3.4 were not included in this study because it has been shown that pH alone is not a determinant of astringency (Lee & Vickers, 2008).

All samples were prepared the day before each test session and stored at 4°C until the day of the test session. The morning of the tests sessions, samples were poured in 2 oz. clear, plastic Solo<sup>®</sup> cups, which were labeled with a three-digit code unique to each sample. Samples were left at room temperature for 1 hour prior to serving in order to equilibrate

them. The order of sample presentations for the test sessions was determined by a Latin Squares design, balanced for carry-over and order effects.

**Test Sessions.** Two test sessions were conducted, each an hour long. The same nine samples were evaluated blind in both test sessions. Prior to tasting any samples, panelists were given a handout of the sampling technique, described in Table 2. An astringency reference was the first sample given at each test session. The reference was a 0.5 g/L alum solution and had an astringency rating equal to 5 on the 15-point scale. Panelists were reminded of the astringency rating that corresponded to the astringency reference and were prompted to wait 1 minute and rinse their mouth with distilled water prior to proceeding to the remaining nine samples. Panelists rated the sourness and astringency of the samples on a 15-point line scale with 0 = not at all astringent/sour and 15 = extremely astringent/sour (Figure 1).



**Figure 1. Line scale used for astringency ratings.** The same line scale was used to collect sourness ratings; however, the word ‘astringency’ was replaced with ‘sourness’.

After rating the sourness and astringency of each sample, panelists were prompted to rinse their mouths with water and wait 1 minute before proceeding to the next sample. Panelists were instructed to extend the waiting period if any astringency was still

perceived in the mouth after the mandatory 1 minute of waiting. Panelists used SIMS 2000 to record their astringency and sourness ratings for all nine samples.

**Data Analysis.** An analysis of variance procedure (ANOVA) was performed in XLSTAT® - Pro (Version 2012.5.01, Addinsoft, Inc., New York, NY, USA) to determine whether any of the samples differed in astringency or sourness. The astringency and sourness ratings were used as the dependent variables. The solution type and order were set as fixed effects, and the judges were treated as random effects. Tukey HSD tests were performed to determine which samples differed from each other in sourness and astringency.

## Results

**Astringency.** As shown in Table 3, M4, the WPI-containing sample titrated with malic acid, had the highest astringency rating out of the WPI-containing samples; however it was not more astringent than P4 or T4. M4 was the only WPI sample as astringent as the phosphoric and malic acid controls (P, M). Alternatively, the HCl-WPI sample (H4) was the only WPI sample less astringent than M4. Astringency ratings did not differ amongst the acid control samples. As seen in studies conducted by Beecher (2006) and Vardhanabhuti et al. (2010), the phosphate control sample (PC) was less astringent than all other samples, indicating that phosphates are not a main contributor to the astringency. In fact, samples P, P4 and PC all had equal amounts of phosphate, yet different astringency ratings. This finding supports Lee & Vickers' (2008) assertion that



astringency of whey protein beverages is caused by acidity and challenges Vardhanabhuti et al.'s (2010) claim that whey protein is the main source of astringency in acidified whey protein beverages.

Unlike the results presented in Table 3, Rubico & McDaniel (1992) found that inorganic acids were more astringent than organic acids. This finding was corroborated by Straub (1992), who found that HCl was the most astringent acid compared to a number of organic acids. This theory however, is still in question, as Thomas & Lawless (1996) who compared citric, malic, hydrochloric and phosphoric acids at three concentration levels, found no differences in the acids' perceived astringencies. Additionally, the results presented here show no differences in astringency among the four acid control samples. The discrepancy in results from the studies may be explained by variations in methods. Rubico & McDaniel (1992) used a trained, expert panel who evaluated the samples using free-choice profiling. Panelists did not have a standard sampling technique to assess astringency, such as the technique used in this study (see Table 1). Additionally, out of 15 samples, only two were inorganic acids; therefore leaving possibility for a context effect, where the differences in sensory attributes between the inorganic and organic samples were more different than sensory differences within the organic samples, thereby skewing attribute ratings. Straub (1992) also used a trained, expert panel and had them assess the samples using magnitude estimation and time-intensity scaling. Between eight and ten panelists were used, which is a small sample size for such methods and consequently led to large variance among the panelists. Also,

as seen in Rubico & McDaniel's study, no standardized sampling technique was established for evaluating astringency, which could increase the potential for panelists to confuse sourness and astringency when rating a sample. In comparison, our study included a larger sample size, thirty panelists, and used a standardized sampling technique for astringency. Acids used in the study were balanced equally between inorganic and organic. Consequently, our study presents reasonable evidence that inorganic acids are not more astringent than organic acids at the concentrations that we used.

**Table 3. Mean Astringency Ratings**

Sample ID	Sample Description	Mean Astringency Rating	Statistical Groups when $\alpha = .05^*$				
T	Tartaric Acid Control	8.6	A				
H	HCl Control	8.1	A				
P	Phosphoric Acid Control	7.7	A	B			
M	Malic Acid Control	7.3	A	B			
M4	4% WPI with Malic Acid	6.6		B	C		
P4	4% WPI with Phosphoric Acid	5.8			C	D	
T4	4% WPI with Tartaric Acid	5.6			C	D	
H4	4% WPI with HCl	5.3				D	
PC	Phosphate Control	1.5					E

\* Samples with the same letters did not differ significantly in astringency

**Sourness.** Table 4 shows the mean sourness ratings for the samples. For the acid control samples, tartaric and phosphoric (T and P) were the most sour samples, followed by malic and hydrochloric (M and H). As for the whey protein samples, the phosphoric acid sample (P4) was the most sour, followed by the malic acid sample (M4). The least sour

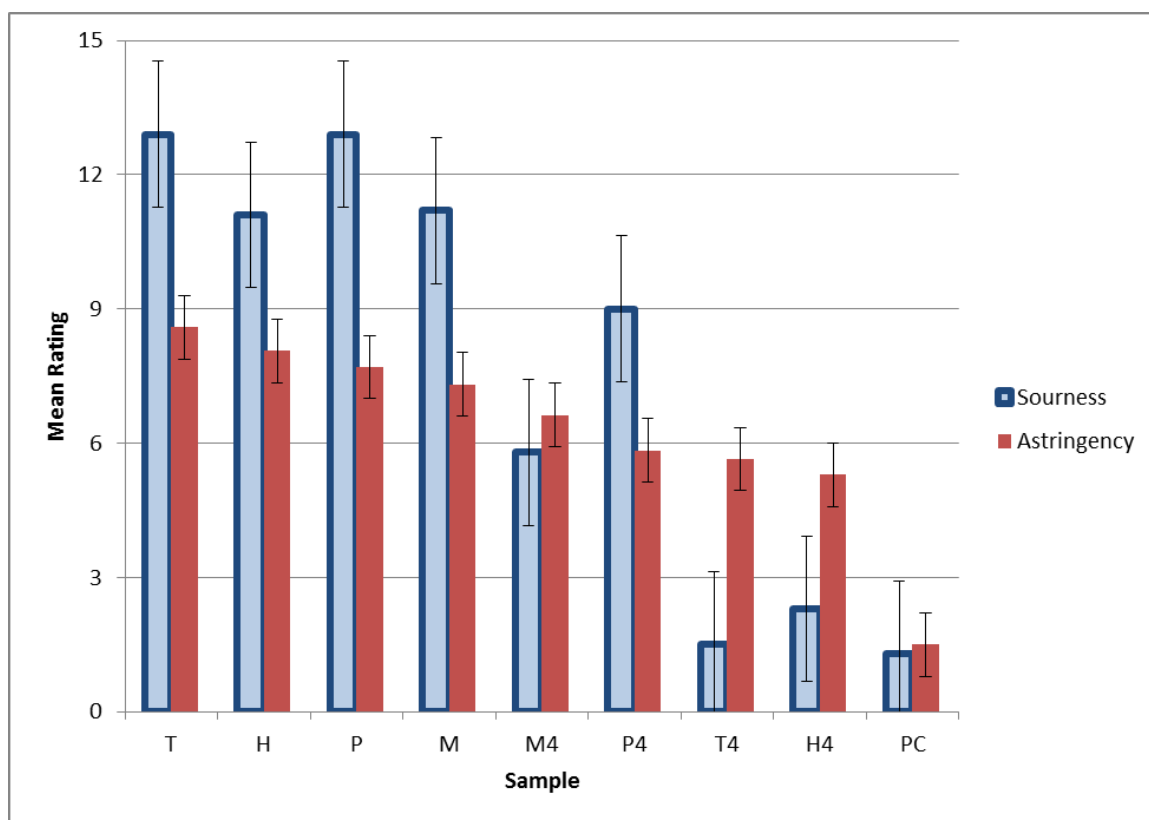
samples were the hydrochloric and tartaric WPI samples and the phosphate control (H4, T4, PC).

**Table 4. Mean Sourness Ratings**

<b>Sample ID</b>	<b>Sample Description</b>	<b>Mean Sourness Rating</b>	<b>Statistical Groups when <math>\alpha = .05^*</math></b>				
T	Tartaric Acid Control	12.9	A				
P	Phosphoric Acid Control	12.9	A				
M	Malic Acid Control	11.2		B			
H	HCl Acid Control	11.1		B			
P4	4% WPI with Phosphoric Acid	9.0			C		
M4	4% WPI with Malic Acid	5.8				D	
H4	4% WPI with HCl	2.3					E
T4	4% WPI with Tartaric Acid	1.5					E
PC	Phosphate Control	1.3					E

\* Samples with the same letters did not differ significantly in sourness

Figure 2 on the following page allows for an easy comparison of each sample's mean astringency and sourness ratings. Among the WPI samples tested, T4 and H4 appeared to have both the lowest sourness and astringency ratings.

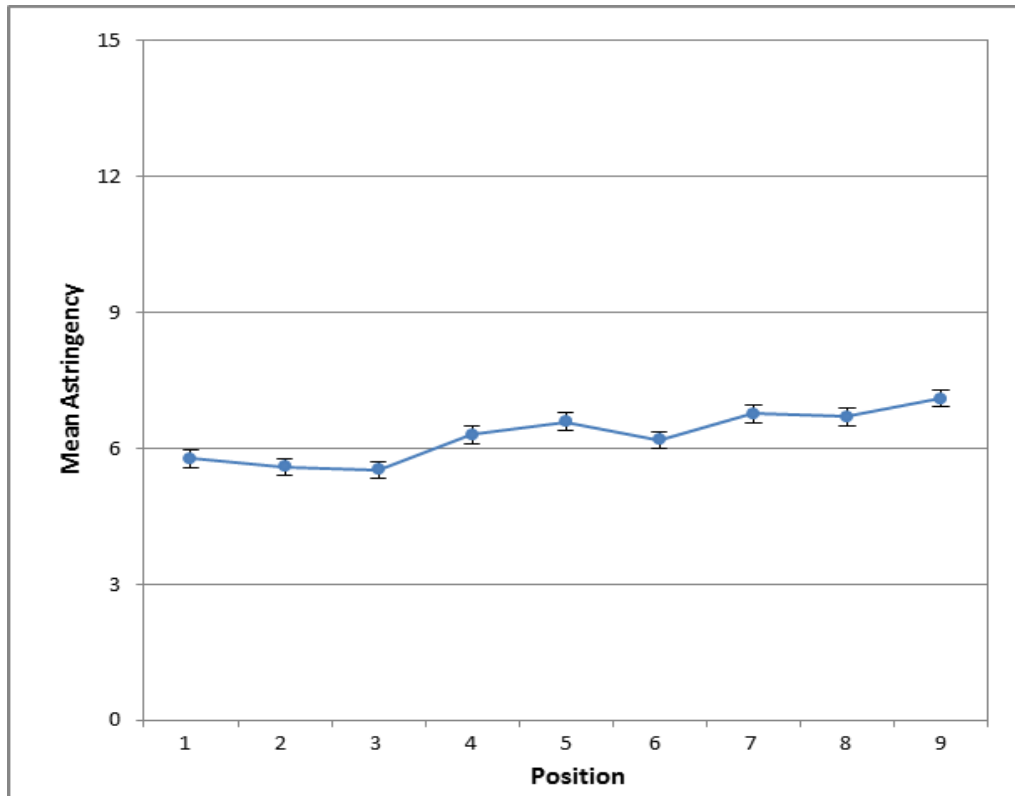


**Figure 2. Mean sourness and astringency ratings for each test sample.** Full sample names can be found in Tables 3 and 4. Thirty panelists rated sourness and astringency of the samples using a 15-pt line scale, with 0 = not at all astringent/sour and 15 = extremely astringent/sour as end points. Error bars represent standard errors.

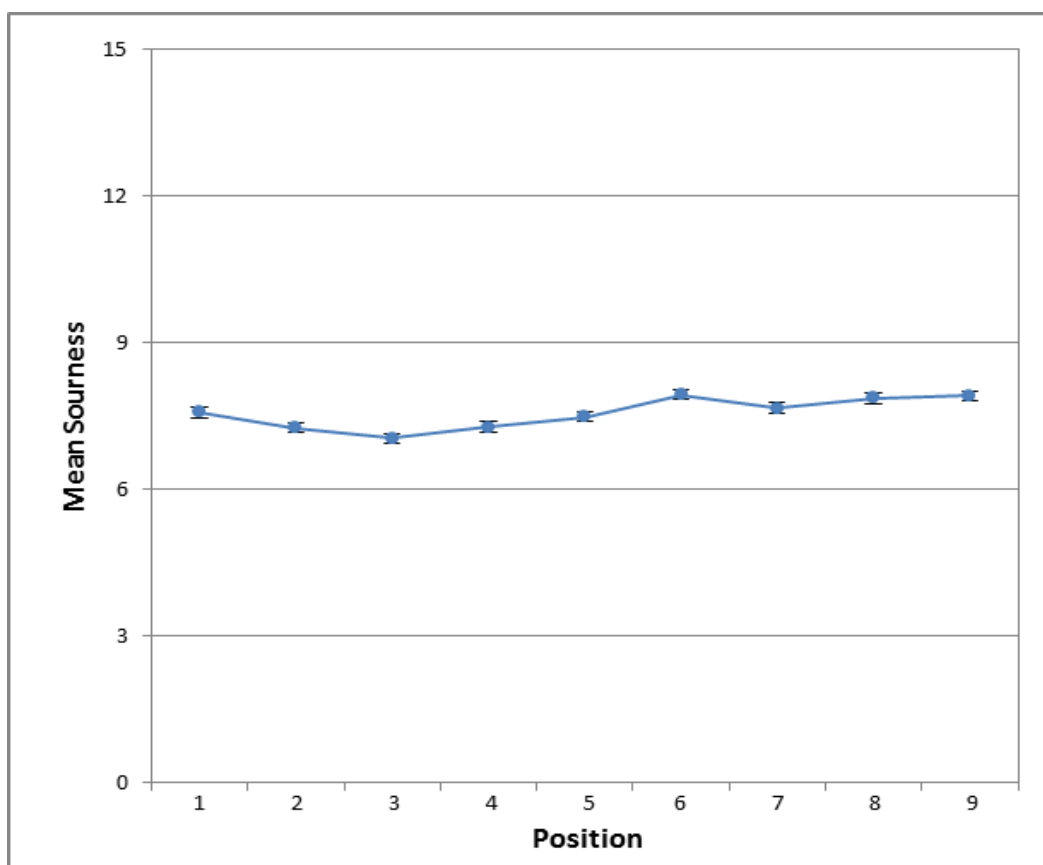
The sensation of astringency is one that can build or intensify with repeated sips of an astringent compound. This trend has been documented for red wine, soy milk and a variety of phenolic compounds (Colonna, 2004; Ross, 2007; Monteleone, 2004).

Consequently, when panelists are asked to evaluate astringent samples, it is typical to see carry-over effects from one serving position to another. In this study, the mean astringency in the last position (position 9) was higher than the mean astringency in the first, second and third positions (Figure 3); however no position effects were found in the

sourness data (Figure 4). Although position effects can skew data, measures were taken in this study, such as forced wait-time between samples and balancing sample order, in efforts to mitigate the impact of carryover. The sensory community has yet to identify an effective palate cleanser to prevent sensory fatigue and carryover effects from occurring, therefore until a solution has been found, order effect is a phenomenon that should be assumed and planned for when designing any test which includes fatiguing stimuli.



**Figure 3. Mean astringency ratings vs. taste position for all test samples and sessions.** Thirty panelists rated astringency of the samples using a 15-pt line scale, with 0 = not at all sour and 15 = extremely sour as end points. Error bars represent standard errors.



**Figure 4. Mean sourness ratings vs. taste position for all test samples and sessions.**

Thirty panelists rated astringency of the samples using a 15-pt line scale, with 0 = not at all astringent and 15 = extremely astringent as end points. Error bars represent standard errors.

### **Buffer Capacity**

#### **Materials & Methods**

Lee and Vickers (2008) have determined that astringency of acidified beverages increases as the beverage's acidity increases. When producing acidified whey protein beverages, a certain pH must be reached; therefore the stronger the acid's buffer capacity within the starting and ending pH range, the more acidic the beverage becomes. In

efforts to determine if, within the pH range of interest (3.4-7.0), a particular acid exhibited a lower buffer capacity than others, malic, hydrochloric, tartaric and phosphoric acids (0.5 molarity) were titrated with 0.1M sodium hydroxide (NaOH) and buffer capacities were calculated using the following equation:  $\beta = \Delta c_B / \Delta pH$  with  $\Delta c_B = c_{NaOH} \times V_{NaOH} / V_{total}$ , where  $\beta$  = buffer capacity,  $\Delta c_B$  = normal concentration of added base,  $c_{NaOH}$  = concentration of added NaOH,  $V_{NaOH}$  = volume of added NaOH and  $V_{total}$  = volume of the buffer mixture and the volume of added NaOH. Additionally, a similar process was followed for a 6% WPI solution. The 6% WPI concentration was selected because this appeared to be the maximum protein concentration found in commercial products at the time of the study. The solution was created using the same method as for the test samples; 1.2g WPI was dissolved in 20 mL water with sucralose and raspberry flavoring. The solution was made four times, and each solution was titrated with a different acid (hydrochloric, malic, phosphorous, tartaric) until a pH between 1.0 and 2.0 was reached. Buffer capacity was then calculated for each of the solutions using the equation stated above; however, the concentrations and volumes of the acids were used in place of the base ( $\beta = \Delta c_A / \Delta pH$  with  $\Delta c_A = c_{acid} \times V_{acid} / V_{total}$ ). Buffer capacity curves were created by plotting the buffer capacity values against the corresponding pH values for each solution (Van Slyke, 1922).

## Results

The whey protein solutions tested in this study started at a pH of approximately 7.0 and were acidified to pH 3.4. From Figure 5, it appears that hydrochloric and phosphoric

acids buffer the least amount within the pH range of interest, 3.4 -7.0, although phosphoric acid does appear to buffer between pH 6.0 – 7.5 and pH 1.0 – 3.0, while hydrochloric only buffers from pH 1.0 – 2.0. Alternatively, tartaric acid and malic acid steadily increase in buffering from pH 6.0 to 3.0. Buffer capacity is closely related to the dissociation constants (pKa) of a compound. When titrated, a solution is likely to buffer at pH levels that match the dissociation constants of its components; however, as a matrix becomes more complex, so does the buffering mechanism. In complex matrices, multiple interactions can take place during titration, thereby affecting the buffering capacity of a solution at a given pH. In regards to this study, the pKa values of the whey protein and acids need to be considered. The literature pKa values of the acids are listed in Table 5 and are similar to the points of maximum buffering identified in this study (Figure 5):

**Table 5. Acid pKa Values**

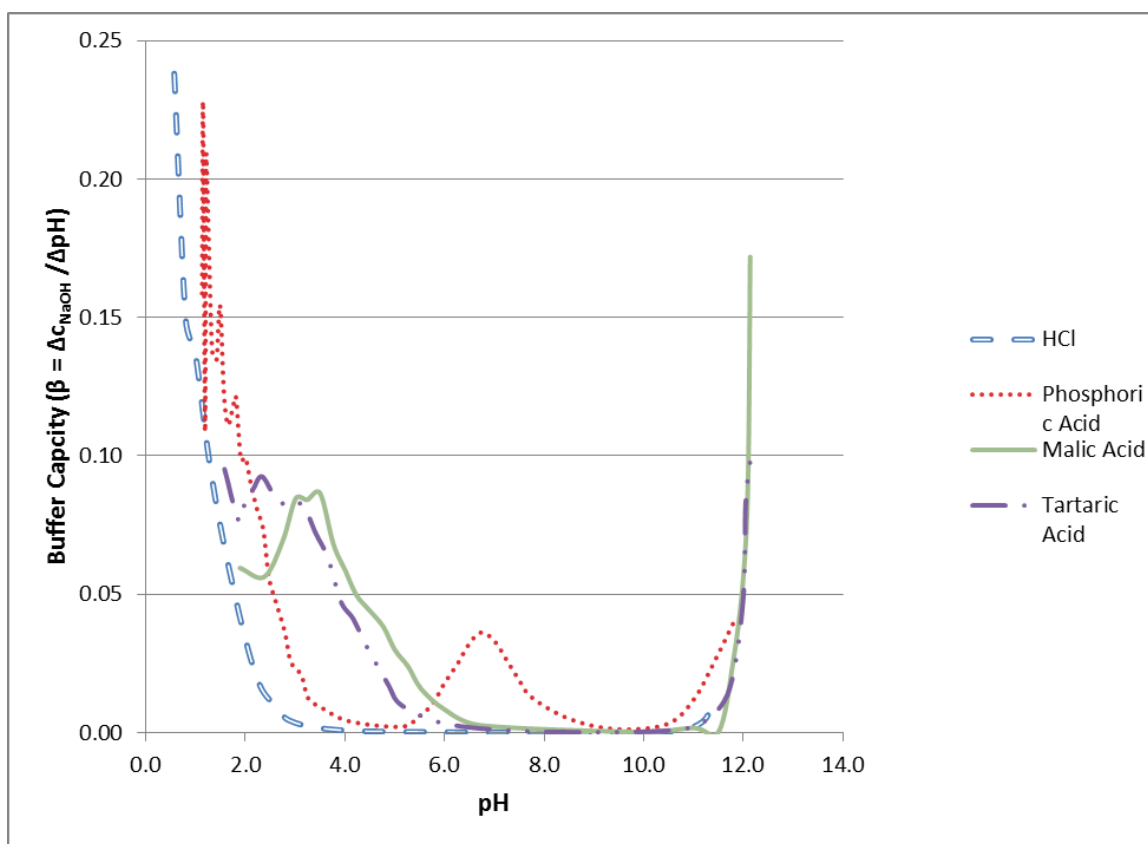
Acid	pKa
Tartaric	2.9, 4.4
Phosphoric	2.1, 7.2
Malic	3.4, 5.0
HCl	1.0

(Mohan, 2003; Sigma-Aldrich, 2004; Sortwell, 2004)

Figure 5 suggests that phosphoric acid and hydrochloric would produce the least acidic solutions, therefore leading to the least astringent solutions amongst the four acids, but as shown in Table 3, astringency was not different among the acid control samples.

However, similarities in perceived astringencies between hydrochloric and phosphoric acids, as suggested by Figure 2, have previously been reported by Thomas & Lawless (1996) and Rubico and McDaniel (1992).





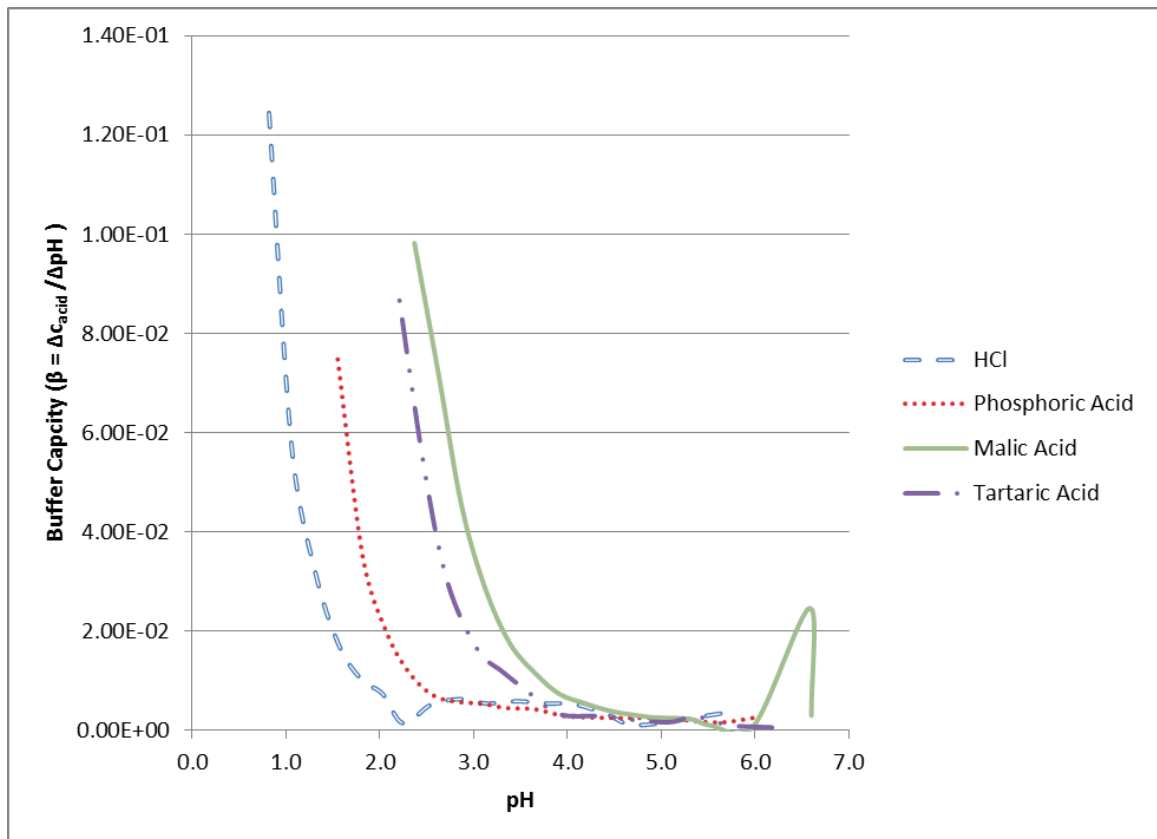
**Figure 5. Buffer Capacity Curves of Acids.** Buffer capacities were determined experimentally and calculated using the equation listed on the ordinate axis.

Figure 6 displays the buffer capacity curves of each of the four WPI-acid solutions.

Compared to the acid-only samples, the addition of WPI appears to narrow the pH range that the solutions buffer within. The HCl-WPI solution buffers between pH 1.0-2.0, the phosphoric acid-WPI solution buffers between pH 2.0 – 2.5, the malic acid-WPI solution buffers between pH 3.0 - 4.0 and 6.0 – 6.5, and the tartaric-WPI solution buffers between pH 2.0 – 4.0. Whey proteins are reported to have maximum buffering capacity between pH 3.0 and 4.0 due to the presence of acidic amino acids (Salaun et al., 2005).

Comparing Figures 5 and 6, it appears that within pH range 2.0-4.0, the buffering

capacity of whey protein increased the buffering rates of malic and tartaric acid solutions, but not hydrochloric or phosphoric acid solutions. Two notable observations from this graph: (1) as shown in Figure 5, hydrochloric and phosphoric acids buffer the least between pH 3.4 and 7.0, followed closely by tartaric acid, and therefore should produce the least astringent beverages, (2) for all acids, the buffer capacities are much lower when acidifying the 6% whey protein solutions than when NaOH was titrated into the acids. Because buffer capacity has no unit, the latter observation simply indicates that it takes less acid to produce a change in pH for the WPI solutions than it takes NaOH to produce a change in pH for the acid-only solutions.

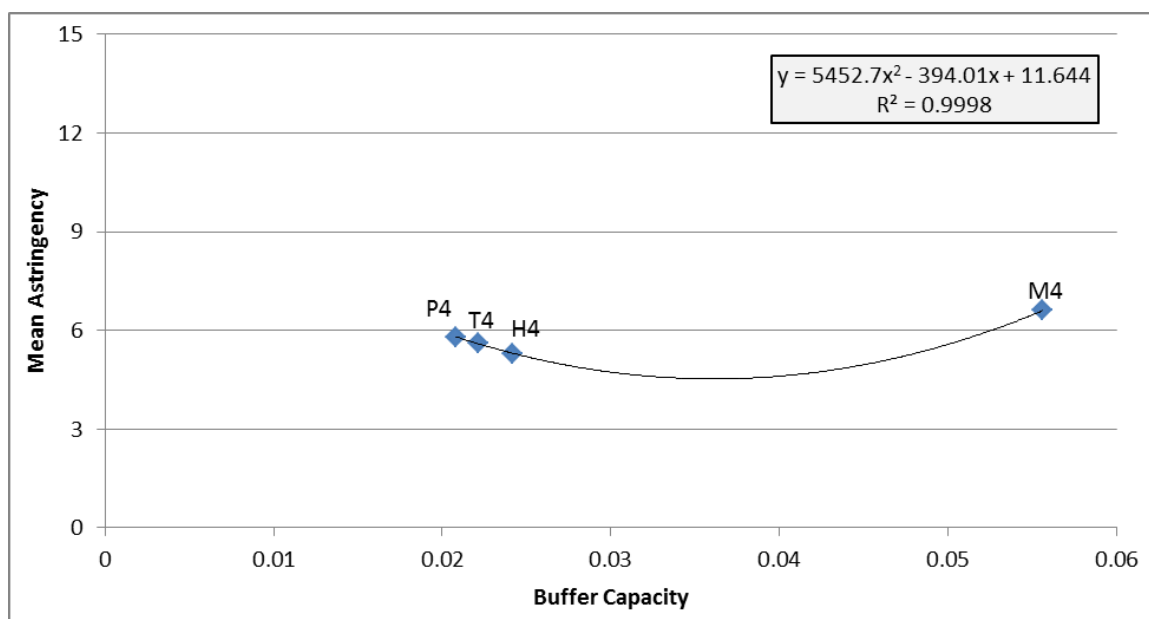


**Figure 6. Buffer Capacity Curves of WPI-Acid Solutions.** Buffer capacities were determined experimentally and calculated using the equation listed on the ordinate axis.

## **Discussion**

### **Astringency**

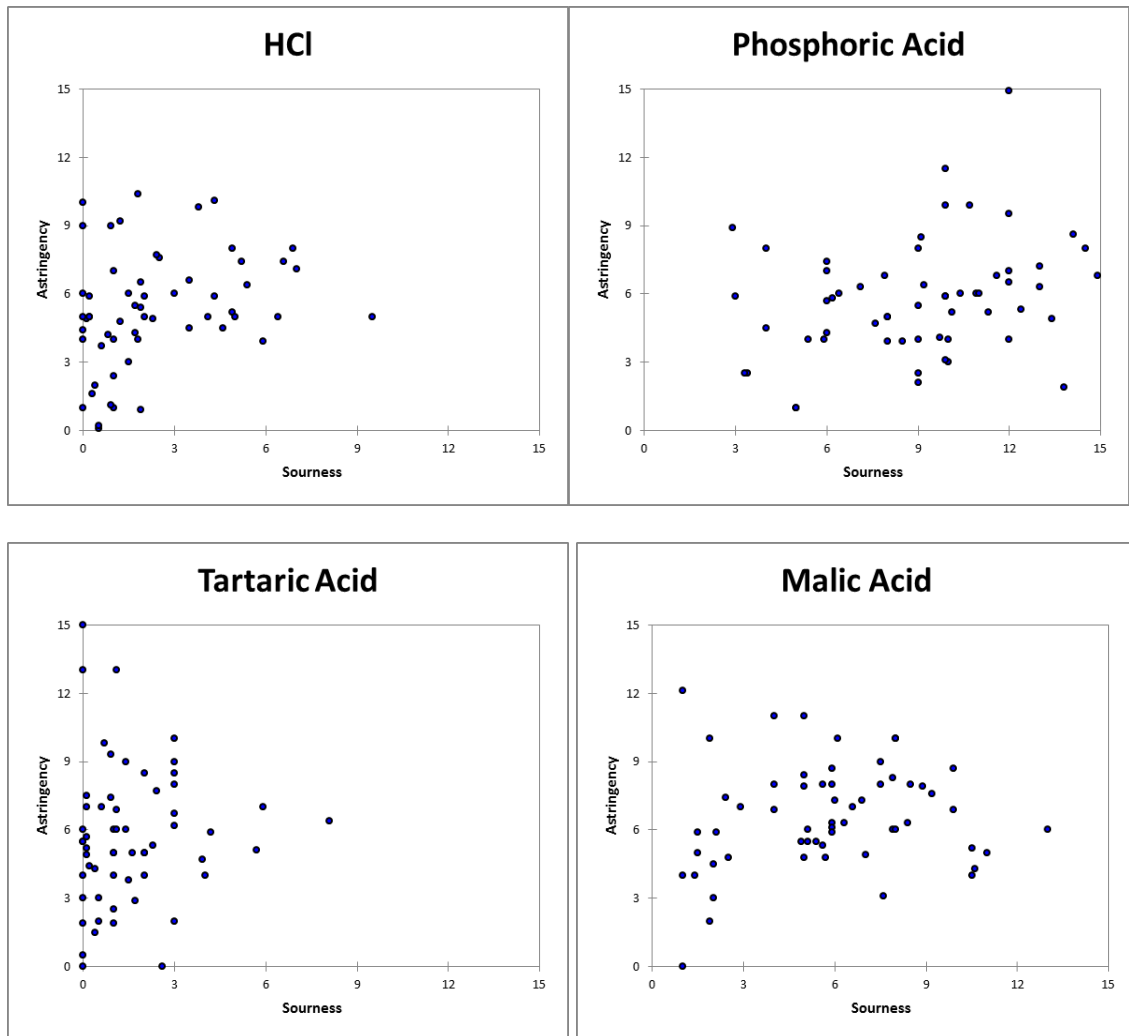
The data indicate a relationship between the perceived astringency of an acidified-WPI solution and the buffer capacity of the solution. More specifically, as shown in Figure 7, the relationship appears to be quadratic ( $y = 5452.7x^2 - 394.01x + 11.644$ ), with astringency decreasing as buffer capacity increases from ~0.020 to ~0.035, and then increasing again as buffer capacity increases from ~0.035. This relationship suggests that by determining the buffer capacities of acids over a pH range that spans the initial and final desired pH of an acidified WPI solution, one can determine the relative astringencies of the final WPI solutions. It cannot be ignored that M4 did contain the largest volume of acid, which based on the theory of acidity causing astringency in whey protein beverages proposed by Lee & Vickers (2008), could be a reason that M4 was found to be more astringent than H4. However H4, which had the lowest astringency rating, did not contain the lowest volume of acid, suggesting that when comparing astringency among acids, buffer capacity may be a better predictor. The quadratic relationship established here, however, is based on four data points, and therefore should be used as an indication only. To more firmly establish this relationship, additional research needs to be conducted that would include a greater number of acids, i.e. data points.



**Figure 7. Relationship between Astringency and buffer capacity of acidified-WPI samples.** Buffer capacity values are the sum of buffer capacities for that acid between pH 3.4 and 7.0. Full sample names can be found in Tables 3 and 4. Thirty panelists rated astringency of the samples using a 15-pt line scale, with 0 = not at all astringent and 15 = extremely astringent as end points.

As seen in Figure 8, no relationship between astringency and sourness could be established from the results of our study. This may indicate that the two mechanisms are entirely independent of one another, and therefore one does not impact another. In spite of this, a few observations were made when comparing each acid's astringency and sourness ratings. The WPI sample acidified with HCl was found to be more astringent than sour, which was in agreement with findings from previous research (Thomas & Lawless, 1995; Straub 1992, Rubico). In fact, P4 was the only WPI sample that had a higher perceived sourness than perceived astringency. This result is surprising given that P4 contained the lowest volume of acid compared to the other samples, and as an acid

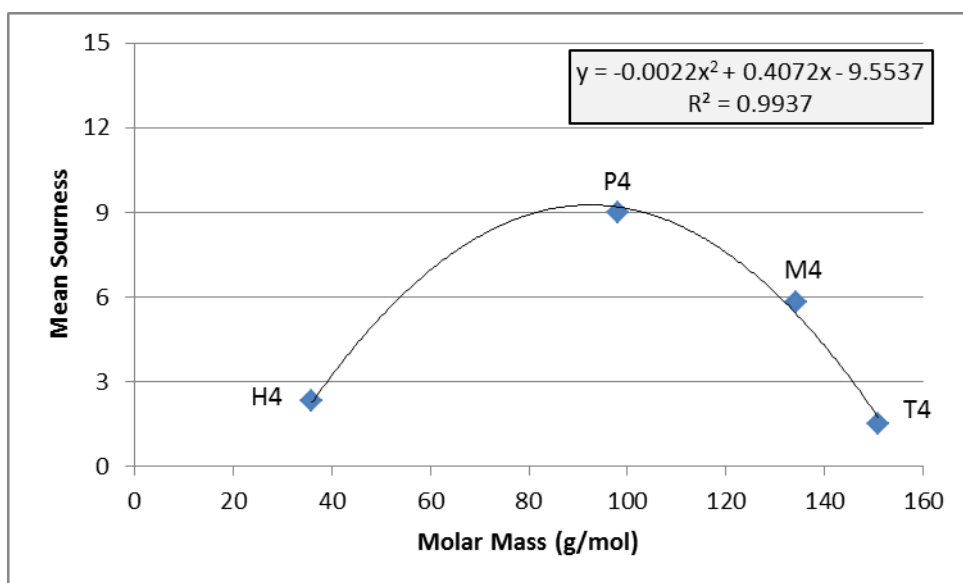
control sample, had the same perceived sourness rating as the tartaric acid control. The high sourness of P4 is an effect of the complexity of the mechanisms occurring in acidified whey protein beverages. Undefined and not well understood, the astringency and sourness mechanisms of acidified whey protein solutions call for further research to be devoted to understanding this important and multifaceted product category.



**Figure 8. Relationship between Sourness and Astringency for each acidified-WPI sample.** Each data point represents each panelist's rating on a 15-pt line scale for sourness and astringency, with 0 = not at all astringent/sour and 15 = extremely astringent/sour as end points.

### Sourness

As previously mentioned, the sourness of the solutions was expected to increase with the molecular weight of the acidulant, therefore tartaric > malic > phosphoric > HCl. This study found some agreement with CoSeteng's et al. (1989) theory; in the acid control samples, tartaric and phosphoric were the most sour and malic and HCl were the least. When the acids were titrated in the WPI solutions, a different order was seen: phosphoric > malic > HCl and tartaric. When plotted, a quadratic relationship was found between the mean sourness of the acidified-WPI solution and the molar mass of the acid (Figure 9). Consequently, molar mass of an acid may be a useful predictor of sourness in acidified-WPI beverages. As mentioned with the relationship between astringency and buffer capacity, this conclusion is based off limited data points, and additional research needs to be conducted with additional acids to more firmly establish this relationship, Future research on understanding this phenomenon should focus on the sourness mechanisms suggested by Ganzevles and Kroeze (1987a).



**Figure 9. Relationship between mean sourness for each acidified-WPI sample and molar mass of acidulant used.** Mean sourness for each acidified-WPI sample is plotted versus the molar mass of the acid used in each sample. Sourness was evaluated by 30 panelists on a 15-pt line scale for sourness with 0 = not at all sour and 15 = extremely sour.

### Conclusion

To minimize the astringency and sourness associated with acidified whey protein beverages, I recommended to acidify the whey protein solution with hydrochloric acid. Malic acid is not recommended due to a higher perceived astringency and sourness ratings. When assessing the potential astringency of acidulants, it is recommended to determine and compare the buffer capacities of the acidulants, as this study suggests perceived astringency increases with buffer capacity.

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